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Discovery of novel 1*H*-imidazol-2-yl-pyrimidine-4,6-diamines as potential antimalarials

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ABSTRACT

A novel family of 1*H*-imidazol-2-yl-pyrimidine-4,6-diamines has been identified with potent activity against the erythrocyte-stage of *Plasmodium falciparum* (*Pf*), the most common causative agent of malaria. A systematic SAR study resulted in the identification of compound **40** which exhibits good potency against both wild-type and drug resistant parasites and exhibits good in vivo pharmacokinetic properties.

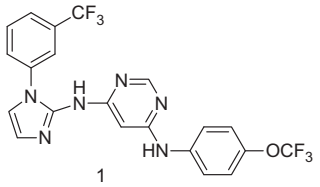
Malaria infection infects nearly 500 million people causing nearly 1 million deaths per year, with highest mortality among pregnant women and young children, despite the recent introduction of artemisinin-based combination therapies (ACTs) as first line therapy.[1,2] Reports of increased drug tolerance to ACTs along the Thai–Cambodian border[3] as well as widespread resistance to quinoline based therapies mandates the development of novel therapies against *Plasmodium falciparum* (*Pf*) parasitic infections.[4] Here we report our discovery of novel 1*H*-imidazol-2-yl-pyrimidine-4,6-diamines with potent activity against the both wild-type and drug resistant parasite strains of *Pf*.

In our efforts to identify new molecular scaffolds structurally unrelated to known antimalarials that could either target parasite or host processes, we performed a cell-based proliferation assay against the erythrocyte stage of *Pf*.[5–7] We screened a collection of kinase inhibitor scaffolds, primarily developed against human kinases with the idea that host cell kinases or nucleotide requiring parasite enzymes could serve as potential targets. Here we report on our medicinal chemistry efforts starting from screening 'hit' **1** (Fig. 1) which possessed a moderate EC_{50} of 436 nM against the chloroquine sensitive 3D7 parasite strain.

Compound **1** appeared to be an attractive lead because it is structurally unrelated to known antimalarials and did not exhibit significant activity when screened against a panel of 40 mammalian kinases suggesting that it is not a promiscuous kinase inhibitor.

In order to systematically investigate the structure–activity relationships with respect to proliferation of *Pf* in erythrocytes, we developed a variety of synthetic routes to this structure (Schemes 1–4). The first route starts with the reaction of variously substituted anilines with cyanamide under acidic conditions to afford the corresponding substituted phenyl guanidine nitrates.[8] Cyclization was accomplished by reaction with 2-chloroacetaldehyde to afford the substituted phenyl-1*H*-imidazol-2-amines as key intermediates. Final products were obtained by sequential acid or base-catalyzed displacement of dichloro-pyrimidines or dichloro-quinazoline with the imidazol-2-amine and a second amine nucleophile of choice.

In order to diversify the phenyl appended to the imidazoyl N1-position, palladium catalyzed amination were performed on



1

3D7 EC_{50} = 436 nM

Figure 1. Imidazolyl pyrimidine screening hit compound **1**.

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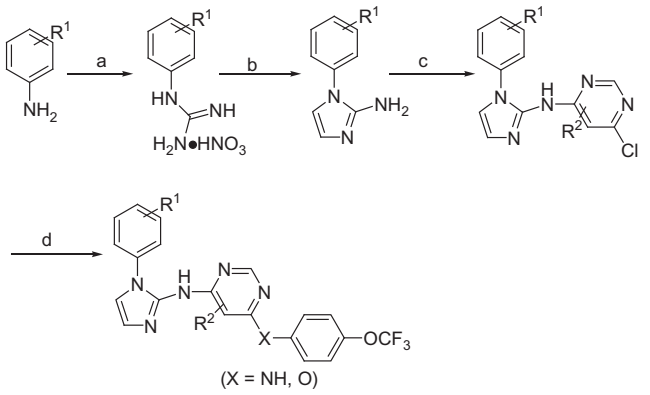
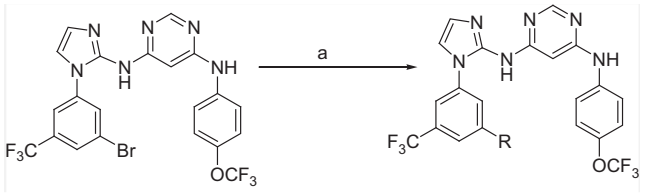


Scheme 1. General synthesis scheme of phenyl-1*H*-imidazol-2-yl-pyrimidine-diamines. Reagents and conditions: (a) NH_2CN (2.4 equiv, 50% aq), concd HNO_3 (1.1 equiv), EtOH, 100 °C, 78–86%; (b) $ClCH_2CHO$ (2.0 equiv, 50% aq), Na_2CO_3 (satd aq), EtOH, 80 °C, 60–85%; (c) substituted di-chloro pyrimidines or di-chloroquinazoline, DIEA, dioxane, 100 °C, 60–80%; (d) 4-(trifluoromethoxy)benzenamine, TFA, 2-PrOH, 80 °C, 78–85%; or 4-(trifluoromethoxy)phenol, NaH, dioxane, rt to 80 °C, 75%.



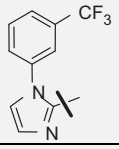
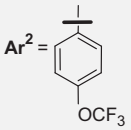
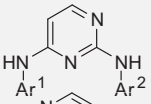
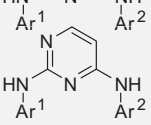
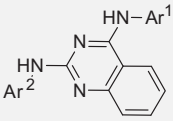
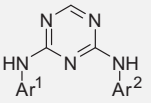
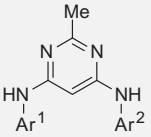
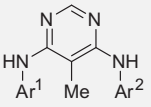
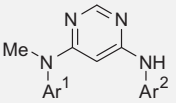
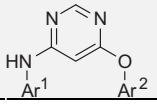
Scheme 2. General synthesis of amine-substituted phenyl-1*H*-imidazol-2-yl-pyrimidine-4,6-diamines. Reagents and conditions: (a) secondary amine, $Pd(OAc)_2$ (10%), BINAP (15%), *t*-BuONa (3.0 equiv), toluene, 100 °C, 50–75%; or primary amine, $Pd_2(dba)_3$ (10%), BINAP (15%), *t*-BuONa (3.0 equiv), toluene,100 °C, 50–70%.

arylbromide containing substrates (Scheme 2) or amide bond formation using arylcarboxylate containing substrates (Scheme 3).[9]

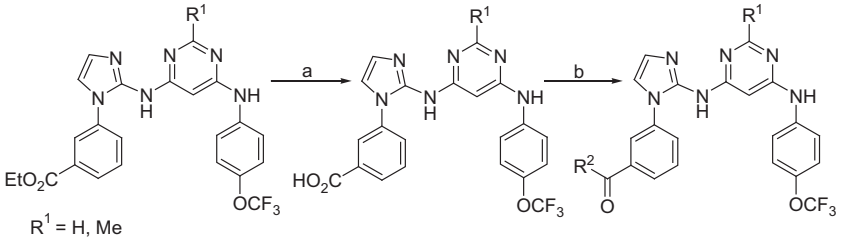
Introduction of functionality at C2 of the central pyrimidine core was accomplished by S_NAr substitution of a C2-methylsulfonyl group with various amines or alkyl alcohols (Scheme 4).[10]

The compound potencies were determined in a *Pf* infected human red blood cell assay using Sybr green staining as the readout for parasite proliferation.[5] Our initial SAR investigation was fo-

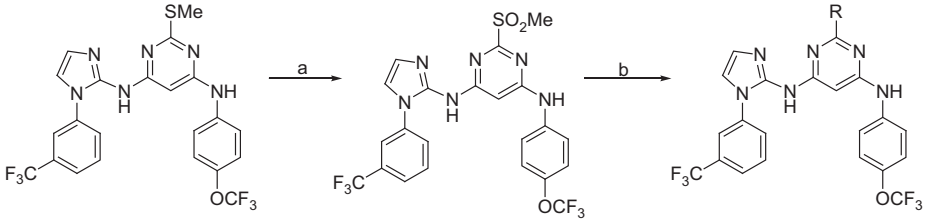
Table 1
SAR of the central pyrimidine core

Compd	Structure	*P. falciparum* 3D7 strain EC_{50}[a] (μM)
	Ar^1 =  Ar^2 = 	
2		0.432
3		0.268
4		0.564
5		1.330
6		0.114
7		4.680
8		3.830
9		4.240

[a] Values are means of two experiments. As internal standards, each assay plate contains mefloquine, sulfadoxine and artemisinin which possess EC_{50} values of 20, 30 and 10 nM, respectively.



Scheme 3. General synthesis of amide-substituted phenyl-1*H*-imidazol-2-yl-pyrimidine-4,6-diamines. Reagents and conditions: (a) LiOH, MeOH/THF/H_2O, rt, 85–95%; (b) primary or secondary amines, HATU, DIEA, DMSO, rt, 75–88%.



Scheme 4. General synthesis of 2-substituted-N^4-(4-(trifluoromethoxy)phenyl)-N^6-(1-(3-(trifluoromethyl)phenyl)-1*H*-imidazol-2-yl)pyrimidine-4,6-diamines. Reagents and conditions: (a) oxone (4.0 equiv), CH_2Cl_2/MeOH/H_2O, rt, 85%; (b) amine, DIEA, dioxane, 80–100 °C, 60–80%; or ROH, NaH, 80–100 °C, 60–70%.

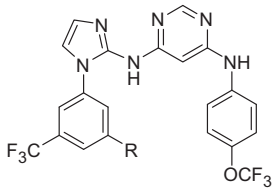
cused on the central pyrimidine core as these modifications were anticipated to dramatically affect the conformational preferences of the inhibitor (Table 1). Replacing the 4,6-pyrimidine of **1** with the two 2,4-pyrimidine regioisomers and 2,4-quinazoline resulted in approximately equipotent compounds **2**, **3** and **4**. The corresponding triazine analog **5** exhibited ~three-fold decrease in potency. Introduction of a methyl group to the pyrimidine C2 (**6**) resulted in an approximate four-fold improvement in potency relative to **1** while a methyl group at the pyrimidine C5 (**7**) resulted in a compound ten-fold less active compared to **1**. Both NH groups of **1** appear to be essential as methylation of the imidazolyl NH (**8**) or replacement of the 4-trifluoromethoxy aniline NH with an oxygen (**9**) resulted in a ten-fold loss of activity.

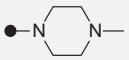
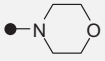
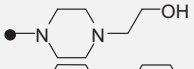
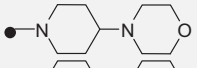
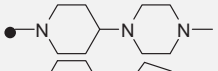
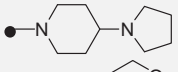
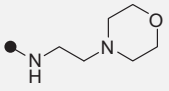
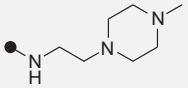
One liability of compound **1** was its very poor aqueous solubility (water solubility of 34 μg/mL at pH 6.8) which we sought to address through the introduction of tertiary amines to the imidazoyl N1 phenyl substituent (Table 2). Introduction of primary, monocyclic and bi-cyclic amine substituents resulted in compounds (**11**, **14, 16, 17** and **18**) that possessed EC_{50} values below 100 nM which represented an improvement relative to unsubstituted compound **1** and bromo-substituted compound **10**.

Since the meta-amine substituent appeared to be favorable and the trifluoromethyl group was not essential (data not shown), we next explored the possibility of incorporating amines linked via one, two or three-carbon spacer to a benzamide amide (Table 3). The most potent compound **22** substituted with a (1-ethylpyrrolidin-2-yl)methan-amide possessed an EC_{50} of 60 nM. Other favorable substituents included ethyl (**23**) and propyl (**27**) morpholinyl containing amides. Histamine amide with a free NH and 2-(piperazin-1-yl)ethanol amide with a free OH resulted in a decrease in potency (compounds **20**, **21** and **25**). Interestingly, we again observed that a C2-methyl substituent on the pyrimidine core could improve the potency (**19** vs **23**, **20** vs **25**).

Based upon the favorable effects of a C2-pyrimidine methyl substitutent, we decided to further explore this position through the synthesis of a focused library (Table 4). Various functionalities were installed, ranging from electron withdrawing groups (**30**, **33**) to electron donating groups (**6**, **32** and **34**) and alkyl substituent (**6**) to aryl substituents (**31** and **36**). Most of them were well tolerated with the exception of the hydroxyl substituent (**37**). Amine substituents increased potency dramatically (**38**–**42**), with optimal activity being obtained for the simple NH_2 (**38**) and piperazine (**40**) substituted compounds which possessed EC_{50} values of approximately 30 nM.

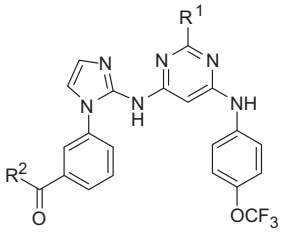
Table 2
SAR of amine-substituted phenyl-1*H*-imidazol-2-yl-pyrimidine-4,6-diamines

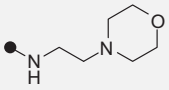
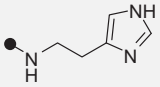
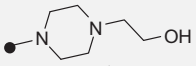
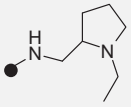
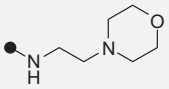
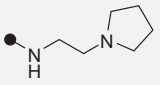
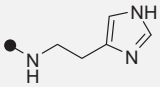
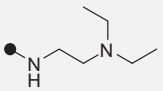
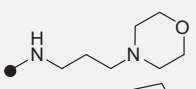
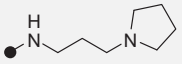
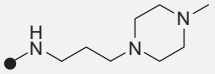


Compd	R	*P. falciparum* 3D7 strain EC_{50}[a] (μM)
10		0.223
11		0.065
12		0.532
13		0.175
14		0.088
15		0.090
16		0.092
17		0.087
18		0.091

[a] Values are means of two experiments. As internal standards, each assay plate contains mefloquine, sulfadoxine and artemisinin which possess EC_{50} values of 20 nM, 30 nM and 10 nM, respectively.

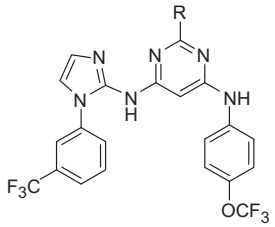
Table 3
SAR of amide-substituted phenyl-1*H*-imidazol-2-yl-pyrimidine-4,6-diamines



Compd	R^1	R^2	*P. falciparum* 3D7 strain EC_{50}[a] (μM)
19	H		0.249
20	H		1.777
21	Me		0.768
22	Me		0.060
23	Me		0.069
24	Me		0.168
25	Me		0.822
26	Me		0.046
27	Me		0.067
28	Me		0.224
29	Me		0.239

[a] Values are means of two experiments. As internal standards, each assay plate contains mefloquine, sulfadoxine and artemisinin which possess EC_{50} values of 20 nM, 30 nM and 10 nM, respectively.

Table 4
SAR of C2-substituent of pyrimidine core



Compd	R	*P. falciparum* 3D7 strain EC_{50}[a] (μM)
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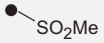
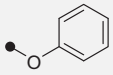
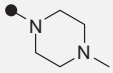
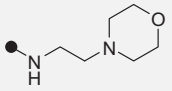
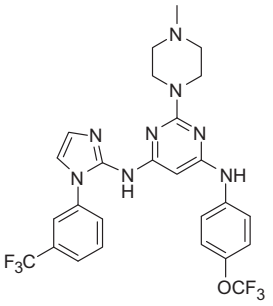
30		0.353
31		0.074
32		0.107
33		0.291
34		0.190
35		0.060
36		0.116
37		5.440
38		0.049
39		0.054
40		0.034
41		0.113
42		0.053

[a] Values are means of two experiments. As internal standards, each assay plate contains mefloquine, sulfadoxine and artemisinin which possess EC_{50} values of 20 nM, 30 nM and 10 nM, respectively.

Table 5
Potencies of compound **40** against 15 *P. falciparum* strains



P. falciparum strain	EC_{50}[a] (μM)
3BAG	0.06
Camp R	0.050
C188	0.14
D10	0.015
D6	0.05
Dd2	0.071
3D7	0.119
FCB	0.05
FCR3	0.139
7G8	0.095
HB3	0.046
K1	0.088
NF54	0.033
TM91C235	0.058
W2	0.12

[a] Values are means of two experiments. As internal standards, each assay plate contains mefloquine, sulfadoxine and artemisinin which possess EC_{50} values of 20, 30 and 10 nM, respectively.

Table 6
Metabolic stability and solubility of selected compounds

Compd	Metabolic stability ER_Mouse[a]	Metabolic stability ER_Rat[a]	Metabolic stability ER_Human[a]	Thermodynamic solubility at pH 6.8[b] (mg/mL)
15	<0.17	ND[c]	0.316	0.11
22	<0.17	0.275	ND[c]	0.089
23	<0.17	0.243	<0.42	0.090
26	<0.17	0.367	<0.31	0.080
40	<0.17	0.264	<0.28	0.040

[a] Values of extraction ratio in mouse, rat or human microsomes.
[b] Values of thermodynamic solubility at pH 6.8.
[c] Not done (assay not performed).

We next evaluated the potency of our new lead compound **40** against a panel of 15 parasite strains (Table 5). The EC_{50} values of compound **40** were all lower than 140 nM, which suggest that this compound may provide a good lead candidate to overcome this set of drug resistant strains. To evaluate a therapeutic index relative to toxicity activity against mammalian cells, compound **40** was tested for antiproliferative activity against a six cell line panel including 293T, Ba/F3, CHO, HEp2, HeLa, Huh7. The EC_{50} values were all greater than 2 μM. As lead compound **1** was originally derived from a kinase inhibitor library, we also tested compound **40** against a panel of 40 cellular tyrosine kinase assays[11] and a panel of 150 biochemical kinase assays at a concentration of 10 μM.[12] This profiling did not reveal significant inhibition of any mammalian kinase.

Some of the more potent compounds were selected to assess their metabolic stabilities and aqueous solubilities (Table 6). Most compounds demonstrated good metabolic stability and exhibited high solubility. Among them, compound **40** exhibited the best metabolic stability with extraction ratio in mouse, rat and human microsome of less than 0.17, 0.264, and 0.28, respectively. An oral and intravenous pharmacokinetic study of compound **40** in mice demonstrated good oral exposure with AUC_{inf} of 14,201 h nM, C_{max} of 946 nM with a half-life of 12 h. The compound was found to have oral bioavailability of 30%.

In summary, we have identified a new imidazolyl pyrimidine scaffold with potent cellular antimalarial activity. SAR studies demonstrated that amine substituent or amide substituent on imidazolyl N1-phenyl ring were favorable. A 4,6-diamine pyrimidine with a free NH appears to be essential for achieving submicromolar potency. A C2-substituent on central pyrimidine core is preferred. The optimized compound **40** displays broad-spectrum activity relative to a panel of 15 parasite strains and displays favorable physiological and pharmacokinetic properties. Our results demonstrate the utility of cell-based screening to identify and optimize new molecular scaffolds possessing antimalarial activity. Further work will be required to establish the molecular target(s) of this compound class and to further investigate their potential as antimalarial drugs.

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